Atty. Dkt. No. 028622-0153

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Alexander LUDEMANN et al.
Title:	METHOD FOR ANALYSING METABOLITES
Appl. No.:	10/580,024
International Filing Date:	12/17/2004
Examiner:	Timothy G. KINGAN
Art Unit:	1797
Confirmation No.	7531

DECLARATION OF DR. LOTHAR WILLMITZER UNDER 37 C.F.R. § 1.132

Sir:

I, Dr. Willmitzer, declare as follows:

1. I am a Professor at the Max Planck Institute of Molecular Plant Physiology. My curriculum vitae is attached as Exhibit A. In relevant summary, I hold a Diplom (1975) and a Ph.D. (1977) in Molecular biology from the Braunschweig Technical University. I have been the director and Scientific Member at the Max Planck Institute of Molecular Plant Physiology since 1994, and hold an honorary Professorship at the University of Potsdam. I have won numerous awards in molecular biology such as The Max Planck Research Award (1994), the DECHEMA prize (1998), the Karl Heinz Beckrus Prize (1999). Prior to my current position, from 1985-1986 I was Leader of an Independent Research Group at the Genetic Research Centre Koln of the Max Planck Institute of Research on Breeding, and in 1986 I was a Professor for Molecular Biology at the University FU Berlin, and Scientific Director of the Institute of Genetic and Biological Research Ltd.

2. I am not being compensated for submitting or preparing this Declaration. I understand that this declaration may be used to support the aforementioned application. Any opinions expressed here are based on my knowledge and experience in the field.

3. I have considerable familiarity with, and specific expertise in, different methods for analyzing the metabolites of biological samples using a variety of techniques. I have published several articles on metabolite profiling including:

a. Rossner *et al.* Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. Plant Cell 13, 11-29 (2001).

b. Urbanczyk-Wochniak *et al.* Parallel analysis of transcript and metabolic profiles: a new approach in systems biology. EMBO Reports 4, 989-993 (2003).

c. Fernie *et al.* Innovation- Metabolite profiling: from diagnostics to systems biology Nature Reviews Molecular Cell Biology 5, 763-769 (2004).

d. Kopka *et al.* GMD.DB: The Golm Metabolome Database. Bioinformatics 21, 1635-1638 (2005).

e. Schauer *et al.* Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. Nature Biotechnology 24, 447-454 (2006).

4. The statements I provide here are based on my knowledge and experience in the field. I understand that my statements will be used in support of the '024 application. I have reviewed the '024 application. I have also reviewed the Office Action mailed October 27, 2010 in the '024 application and the cited references. Specifically, I have reviewed Lee et al. (US Patent Pub 2003/0180710), Abramson et al. (US Patent Pub 2003/0077572), Kasper (US Patent Pub 2005/0112706), Birkemeyer et al (J. Chromatography A 993:89 (2003)), Hellerstein-APEM (American J. Physiol. Endocr. Metab. 276: 1146-1170 (1999)), Hellerstein et al (US Patent Pub 2004/0081994), and Evans (US Pat No 5,532,206).

Lee *et al.*

5. Lee teaches the determination of the dynamic distribution of the label isotope in specific compounds for a mechanistic purpose. Lee uses only one sample (no combination of labelled and unlabelled samples) and measures those samples at specific time points to determine the dynamics of these metabolites. In determining the 13-C/12-C ratio of certain

metabolites Lee does not claim to perform absolute quantification, rather Lee shows how to quantify the relative changes of the labelling ratio.

6. The purpose of Lee's method is to use "smartly labelled" precursors to explore **individual** metabolic pathways. It is not possible to obtain a saturated uniformly labelled sample, independently of the duration of labelling.

7. A uniformly and saturated labelled sample without any further change after being produced is only useful if combined with an unlabeled sample (of natural 13C content) for use as an internal standard for precise, absolute quantification.

8. If Lee were to use uniformly and saturated labelled samples, Lee could not determine the dynamics or pathways involved.

9. Lee's method requires the measurement of time dependent effects to determine the dynamics of the labelled compound.

Abramson *et al.*

10. Abramson teaches the saturated, uniform labelling of a sample and the combination with an unlabelled, but treated, sample for quantification purpose. Abramson does not teach a method for analysing the metabolites of a biological sample which comprises quantitatively determining one or more metabolites in said sample in a way that said quantitative analysis resolves isotopic mass differences of the metabolites themselves or of at least one specific mass spectral fragment per metabolite originating from or being representative for the particular metabolite. Abramson only measures the isotopic mass difference of the combustion products but not the metabolites.

11. Abramson combines an established method, isotope ratio mass spectrometry (IRMS), with chromatography isotope dilution analysis. In IRMS, the sample is usually decomposed to unspecific small compounds, such as CO_2 for all carbon atoms of the sample.

12. Abramson uses CRIMS, a special interface for combustion, to decompose the sample. This type of analysis is a convenient, simple and easy way to apply isotope dilution

analysis, however, the output contains only **two-dimensional data**. The obtained isotope ratio compared against the chromatographic analysis results in the direct quantitative measure for compositional changes of the sample after treatment

13. A limitation of Abramson is based on the combustion process necessary to decompose the sample. All structural information of the metabolites is lost because the sample is decomposed via combustion. Thus, Abramson characterizes metabolites using only chromatographic retention time.

14. Abramson is insufficient for calculating individual quantitative results on thousands of compounds and not suited for complex metabolomic analysis.

15. Abramson combines an ionising mass spectrometer in parallel to CRIMS, but only for analyte identification purposes. Abramson requires a fraction collection step before the analyte can be identified. However, these steps do not improve the poor selectivity and resolution of metabolites for the quantification.

16. The invention described in US 10/580,024 includes an analysis of the molecular and fragment ions masses. This increases the selectivity in a manner so that completely coeluting compounds with known and different mass spectra can easily be resolved. Surprisingly, even partly coeluting metabolites with unknown mass spectra can be resolved by deconvolution. This method allows for the comprehensive, precise and quantitative measurements necessary for metabolomics.

17. I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

5/25/2010

Date

Dr. Lothar Willmitzer

Appendix A

CV Lothar Willmitzer

Personal details

Name, academic title	Lothar Willmitzer, Prof. Dr.
Place and year of birth	Osterburg/Sachsen-Anhalt, Germany, 1952
Nationality	German
Institution and department	Max-Planck-Institute of Molecular Plant Physiology Wissenschaftspark Golm
Postal Address	Am Mühlenberg 1, 14476 Potsdam, Germany
Phone, email	+49 (0) 331 567 8202, Willmitzer@mpimp-golm.mpg.de

Career/Employment

1993 to present	Director of the Max-Planck-Institute of Molecular Plant Physiology and Scientific Member of the Max-Planck-Society
1986	Professor for Molecular Biology at the University FU Berlin, at the same time Scientific Director of the Institute of Genetic and Biological Research Ltd.
1985 - 1986	Leader of an Independent Research Group at the Genetic Research Centre Köln of the Max-Planck-Institute of Research on Breeding

Awards and academic activities

1981	Otto-Hahn-Medal of the Max-Planck-Society
1985	Dozentenprize of the Fonds of the Chemical Industry
1994	Max-Planck-Prize of the Max-Planck-Society
1998	DECHEMA Prize of the Max Buchner Research Foundation
1999	Karl-Heinz-Beckurts-Prize
2002	ISI Web of Sciences "Highly cited researcher"
since 1993	Member of the European Molecular Biology Organization (EMBO)
since 1993	Member of the Berlin-Brandenburg Academy of Science
since 1993	Corresponding Member of the Göttingen Academy of Science
since 1994	Member of the "Academia Europaea"
since 1999	Member of the "Leopoldina", Halle, German National Academy of Science